1. Title of the thesis

"Studies on Hydrolysis of Phosphate Esters and their Bioactivity"

2. Introduction

Phosphate esters are the derivatives of orthophosphoric acid, which can form series of phosphate esters with the combination of alcohols, amines, phenols and their substituted compounds. The chemistry of phosphate esters continues to attract the attention due to their versatile application in various fields. Phosphate esters play most important role in physiology of cells and hence are essential to every organism. Phosphate esters are structural and functional part of DNA, RNA and its monomeric building blocks[1]. It is crucial in transmission of chemical energy through the ATP. Biological phosphate systems are seamlessly linked to life supporting processes such as regulation of blood pH, energy transfer, cell signaling and recognition[2].

There are three types of linkages present in phosphate esters C-N-P, C-S-P and C-O-P. The phosphate ester comprises a group of monoesters, diesters and triesters depending upon the number of hydroxyl groups which are present at phosphorus moiety respectively. Phosphate monoesters and diesters are relatively stable[3]. Monoesters are intermediates in variety of metabolic pathways and diesters have been the subject of numerous theoretical and experimental studies [4]. Triesters are more reactive than the corresponding mono and diesters and do not participate in living organism as metabolites[3, 5].

Phosphate esters have become the subject of research interest to the chemist and biologist. These esters are a class of important compounds similar to pesticides. They have potent insecticidal activities[6]. Phosphate esters are important surfactants. These compounds are important ingredients in high grade detergents, cleaning agents, flame retardants, dispersion agents in paints, primer and metal surface treatments[7]. Synthesis of phosphate esters is an important objective in organic synthesis since they

have many uses in the preparation of biologically active molecules and also versatile intermediates in synthesis of amides and esters[8].

Due to pivotal role these compounds have become therapeutic target in various modern medicines [9]. Most of non polymeric phosphate esters that occur in cell might be considered as lead compounds for the development of drugs[1]. In the recent year few phosphorus esters have received an increasing amount of attention due to significant biological interest and their potential bioactivity against wide spectrum of diseases[10]. Nucleoside derivatives of phosphate ester compounds have found to be extremely important agents for anticancer and antiviral therapy[11].

The hydrolysis of phosphate esters is one of the most fundamental chemical and biochemical reaction[12]. This reaction depends upon both structural and environmental conditions and it is of great interest to the biochemist, environmental and physico-organic chemists. In present investigation an attempt will be made to synthesize some new types of phosphate esters and to study their hydrolysis under the different experimental conditions such as hydrogen ion concentration, ionic strength, solvent effect, temperature effect, substrate concentration, etc. This Study will provide probable reaction mechanism. The determination of mechanism is expected to reveal the possible correlation between reaction paths of chemical and enzymatic hydrolysis of biologically important esters.

There are several microbes in all over the world, which constitute the major portion of biomass in the earth. These microbes can cause many infectious diseases. Due to rapid rise in microbial resistance to traditional antibiotics there is a significant need to discover new types of antimicrobial agents, which reduce microbial growth and cause no harm to the host cell. This goal can be achieved by the discovery of variety of antimicrobial agents[13, 14]. Therefore work will be further extended to study antimicrobial activity of phosphate esters against several microbes with microbial growth kinetics. Microbial growth kinetics with different parameters such as pH, temperature, nutrients concentration will be useful to describe the strength of

antimicrobial activity of new synthesized phosphate esters. This antimicrobial activity of phosphate esters will be useful in the field of medicines and agriculture.

3. A brief review of the work already done in the field

Review of literature summarizes the work which has been done in this field.

Hoque and Lee[15] [2011] have studied the kinetics and mechanism of anilinolysis of diisopropyl chlorophosphate in acetonitrile at 55^0C. Kumar et al.[16] [2010] have reported about synthesis, kinetic behavior and mechanism of hydrolysis of Tri-2,5-diethoxy aniline phosphate (Organophosphate pesticide), in acidic media.

Tunnon and coworkers[17] [2009] have presented the reaction mechanism of phosphate monoester hydrolysis in alkaline phosphatase by means of hybrid QM/MM simulation. Kinetics and mechanism of hydrolysis of mono-2-chloro-5-nitroaniline phosphate via conjugated acid species have been documented by singh and shinde[18] [2008].

Tiago et al.[19] [2007] have studied the intermolecular general acid catalysis of the hydrolysis of 2-(2-Imidazolium) phenyl phosphate. Hengge and Sorensen-Stowell[20] [2006], have investigated the thermodynamic origin of the increased rate of hydrolysis of phosphate and phosphorothioate esters in DMSO/water mixtures.

Whiteside et al.[21] [2006] have estimated the rate constants of phosphate ester alkaline hydrolysis. Zhan and Chen[22] [2004] have performed a series of first principles electronic structure calculations to examine the reaction pathway and corresponding activation free energies for alkaline hydrolysis of representative phosphodiesters. Transition state differences in hydrolysis reactions of alkyl versus aryl phosphate monoester monoanions have been presented by Hengge et al.[23] [2003].

Bhoite et al. [24][2012] have studied minimum inhibitory concentration (MIC) for the determination of novelty and potency of Di-2-ethylaniline phosphate with antibacterial activity by using broth dilution assay. Synthesis of a series of novel phosphorylated derivatives of Zidovudine has been performed by Naga Raju et al.[25] [2010]. They have also studied their antimicrobial activity which is greatly influenced

by the presence of different bioactive groups at phosphorus. Synthesis and antibacterial and antifungal properties of some phosphorus-containing 1,2-dihydroxynapthalenes have been carried out by Khasiyatullina and coworkers [26] [2009]. They have also established the structural-activity relationship.

Reddy et al.[27] [2008] have Synthesized some oxazaphosphinine oxides and evaluated their antimicrobial activity against gram positive staphylococcus aureus and gram negative klebsiella pneumonia by using disc diffusion method. Shingare et al.[28] [2006] have derived α-hydroxyphosphonate and α-acetyloxy-phosphonates and reported their antibacterial activities against gram positive bacteria by using standard Streptomycin.

4. Noteworthy contribution in the field of proposed work

Organophosphates have achieved a great importance owing to their biological and physical properties as well as utility as synthetic intermediates. Phosphate, phosphonate, phosphoramidate functionality are used as prodrug moieties to enhance the water solubility[29]. 1-chloroethyl phosphate and phosphoramidates are excellent building blocks for the synthesis of novel ethylidene-linked phosphate prodrugs[30]. The phosphonoformate trianion ("foscarnet") (PFA) is active against herpes simplex virus and AIDS-related human cytomegalovirus[31]. Some of the phosphorus compounds belonging to nucleotide family such as AZT 5'phosphate and its isoteric phosphonates are good antiviral agents[32]. Inositol + IP_6 (Inositol hexaphosphate) enhances the anticancer effect of conventional chemotherapy controls cancer metastases, and improves quality of life[33]. Clindamycin phosphate is an important medicine. Chemical or enzymatic hydrolysis of clindamycin phosphate is necessary to obtain the antibiotic activity of the clindanycin base.

There are 140 organophosphate compounds being used as pesticides and as plant growth regulators around the world. These compounds are components of more than 100 types of commercially available pesticides[34] (such as Paraoxon, Parathion, Coumaphos and Diazinon). S-esters, O, O-dialkyl phosphorothioate[35,] pyrophosphate

from dimethyl amine phosphate show an effective insecticidal property. Recently few phosphate esters such as Di-2-ethyl aniline phosphate[24], mono-p-toluidine and di-m-toluidine phosphates[36] have been reported as antimicrobial agents. These phosphate esters have been found to be effective against bacteria and exhibited a significant antibacterial activity. Phosphate esters are also commercially important and used in variety of purposes such as, Tributyl phosphates (TBP) as additives in cement and as a carrier for fluorescent dyes, additives for coating and floor finishing[37]. Phosphate esters are important surfactants widely used in chemical fibers, and daily chemical products.

5. Proposed methodology during the tenure of research work

I. Synthesis of phosphate esters:-

Organic phosphate esters will be synthesized by the known method described in earlier literature[38], which involves the reaction of parent compound with phosphorylating agents such as $POCl_3$, PCl_5 and P_2O_5 in different mole ratios.

II. Spectral characterization of synthesized phosphate esters:-

- **Melting point:** Melting point of the synthesized phosphate esters will be noted.
- **Elemental analysis:** Analysis of C, H, N, P elements and comparison of observed percentage of these elements with theoretical percentage will be done.
- **Spectrum analysis:** IR and NMR spectra of synthesized phosphate esters will be recorded and observed frequencies of these functional groups of P=O, C-N, C-H, P-N, O-H, N-H etc will compared with theoretical one.

III. Kinetics of hydrolysis of phosphate esters and spectrophotometric determination of inorganic phosphates:-

Kinetics of hydrolysis of the phosphate esters will be done in detail under different experimental conditions such as effects of hydrogen ion concentration, ionic strength, temperature, solvent effect etc. which govern the rate of hydrolysis.

Progress of kinetic study for the hydrolysis of the phosphate esters will be studied by spectrophotometer, using Allen's modified method[39]. The inorganic phosphate produced during the hydrolysis

$$R\text{-}NH.PO(OH)_2 + H_2O \longrightarrow R\text{-}NH_2 + H_3PO_4$$

(*Inorganic phosphate)*

forms phosphomolybdate complex $(NH_4)_3PO_4.12MoO_3$ with ammonium molybdate solution which reduces into molybdenum blue by the action of 2, 4-diaminophenol dihydrochloride (amidol) and sodium metabisulphite solution. The intensity of blue colour is directly proportional to the amount of free phosphoric acid.

IV. Study of antimicrobial activity with microbial growth kinetics:-

Antimicrobial activity such as antifungal and antibacterial, which are useful in the field of medicine and agriculture, will be tested against several microbes as bacteria, fungi, by using standard antibiotics like penicillin, streptomycin, and tetracycline. Different techniques such as pour plate technique[40], Paper disc diffusion[41] will be used for the determination of antimicrobial activity and spread plate technique[42] for the study of growth pattern of microbes.

6. **Expected outcome of the proposed work**

Different new types of organic phosphate esters will be obtained. The kinetic study of hydrolysis of these esters will provide information about the rate and mechanism of reactions. The study of mechanism has great academic and commercial

values. Academically it may be a demanding task to determine all theoretically possible reaction paths which have basic importance in both chemical and biochemical reaction mechanism. Knowledge of this mechanism will also be helpful in various applied fields as industrial, medicinal and agricultural etc. The information about study of biological properties of phosphate esters in term of their antibacterial and antifungal activities will throw light on their uses in medicines and in the field of agriculture. Thus the study will contribute significantly in area of academic research and applied fields of chemistry.

7. **Bibliography**:

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